

Stockholm, 1963

Thesis



ON ESTIMATING THRESHOLD LIMITS
FOR MERCURY
IN BIOLOGICAL MATERIAL

BY
MATHS BERLIN

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FROM THE INSTITUTE OF HYGIENE AND THE NOBEL INSTITUTE FOR NEUROPHYSIOLOGY,
KAROLINSKA INSTITUTET; THE DEPARTMENT OF GENERAL HYGIENE, NATIONAL INSTITUTE OF PUBLIC HEALTH, AND THE DEPARTMENT OF PHARMACOLOGY, ROYAL VETERINARY COLLEGE, STOCKHOLM, SWEDEN

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This is a discussion and summary of the following eight papers:

BERLIN, M. (1962 a): A pump for use in measuring arteriovenous differences in concentration. *Experientia* 18: 237.

BERLIN, M. (1962 b): Simultaneous measurement of blood flow, glomerular filtration rate and urine secretion in the separate kidneys of anesthetized rabbits. *Acta Physiol. Scand.* 55: 245.

BERLIN, M. (1962 c): Continuous recording of arteriovenous differences in concentration of radioactively labelled substances. *Acta Physiol. Scand.* 56: 82.

BERLIN, M. (1963): Renal uptake, retention and excretion of mercury, II. A study in the rabbit during infusion of methyl- and phenylmercuric compounds. *Arch. Environmental Health*.

BERLIN, M. and GIBSON, S. (1963): Renal uptake, retention and excretion of mercury, I. A study in the rabbit during infusion of mercuric chloride. *Arch. Environmental Health*.

BERLIN, M. and ULLBERG, S. (1963 a): Accumulation and retention of mercury in the mouse, I. An autoradiographic study after a single intravenous injection of mercuric chloride. *Arch. Environmental Health*.

BERLIN, M. and ULLBERG, S. (1963 b): Accumulation and retention of mercury in the mouse, II. An autoradiographic comparison of phenylmercuric acetate with inorganic mercury. *Arch. Environmental Health*.

BERLIN, M. and ULLBERG, S. (1963 c): Accumulation and retention of mercury in the mouse, III. An autoradiographic comparison of methylmercuric dicyandiamide with inorganic mercury. *Arch. Environmental Health*.

Reference will be made to these papers using the years and letters as listed above in brackets.

ACKNOWLEDGEMENTS

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Stockholm, April 1963.

Maths Berlin

INTRODUCTION

Poisoning by mercury was described in China as long ago as 754 B. C. and two hundred years later in India as well. The first known report of intoxication arising from the occupational use of mercury was given by Paracelsus on the basis of observations among the cinnabar miners at Idria (cited by ALMKVIST, 1933). The appearance, in 1700, of Bernardo Ramazzini's "*De morbis artificum diatriba*" did much to elucidate the symptomatology of chronic mercury poisoning, being derived from a comprehensive study of illness among workers in the quicksilver mining, goldplating and mirror-silvering industries, and among lay healers of that day whose practice consisted in the application of mercury ointment. Ramazzini's treatise was followed by several others in a similar vein; these have all been reviewed by ALMKVIST. The next important advance in our knowledge of industrial mercury intoxication was due to Adolph Kussmaul whose "*Untersuchungen über den konstitutionellen Merkurialismus*" was published in 1861 and summarized his observations, comprising 210 cases of chronic mercury poisoning, among workers at Fürst and Erlangen. Kussmaul pointed out, among other things, the difference between chronic intoxication and the acute variety, which was encountered as an undesirable consequence of the therapeutic

use of mercury. Several other studies followed, based on material from different industrial sites where exposure to mercury occurred; these have been reviewed by NEAL *et al.* (1941), SPIEGL (1957) and AXELSSON & FRIBERG (1960).

In contemporary industrial practice, the use of mercury and its compounds is widespread. The element is employed, for example, in various physical instruments and in electrical relays, and in electrolytic cells and processes. The inorganic salts of mercury find use as catalysts in several chemical processes, and in the fur and felt hat industries. Several organic compounds of mercury, because of their fungicidal and bactericidal properties, have been used as seed dressings and as preservatives for such organic materials as wood and wood pulp. In the past twenty years, a number of cases of occupational poisoning have been caused by these mercurials (HUNTER *et al.*, 1940; AHLMARK, 1948; COTTER, 1947). It has been proposed by several European and American authorities that the maximum allowable concentration (M.A.C.) in air for inorganic mercury, as defined by the International Symposium on Maximum Allowable Concentration Values in Prague 1959, be 0.1 mg/m^3 air, although Russian investigators have recommended a value considerably lower, i.e. 0.01 mg/m^3 air. An

M.A.C. value of 0.01 mg/m^3 air has been suggested for all organic mercurials.

AXELSSON & FRIBERG (1960) have reviewed the experiments and experience upon which were based the M.A.C. values suggested and applied in recent years. They concluded that the evidence in the literature did not permit more than a rather inexact estimate of the risks involved in occupational exposure to inorganic mercury, and that an M.A.C. of 0.1 mg/m^3 air was probably too high. They also pointed out that for organic mercurials, a scientific basis for the estimation of occupational hazard was lacking but that there were strong reasons to assume that the risk in chronic exposure could differ considerably between organic and inorganic compounds of mercury.

The urinary excretion of mercury has been used as an index in man of the degree of exposure to mercury and of the amount absorbed and retained in the body. TURRIAN *et al.* (1956), however, could not find any correlation between urinary excretion of mercury, clinical signs and level of exposure in a study of 58 workers exposed to mercury. They concluded

that an excretion of more than $300 \mu\text{g}$ mercury per litre urine corresponds to an exposure considerably exceeding the M.A.C. value of 0.1 mg/m^3 air. AXELSSON & FRIBERG (1960) have suggested that the lack of congruence between excretion and exposure shown in previous clinical investigations may be due, in part, to the difficulties in obtaining exact data in factory investigations of the level of exposure. They expected that if more accurate data were obtained, a better correlation could be demonstrated.

In view of the above, it would be desirable, first, to establish a scientific basis for the determination of M.A.C. values for mercury and its compounds, and second, to find a reliable index of the degree of body retention of mercury which could be used in diagnosis and prophylaxis. To contribute to the achievement of these aims, the author has conducted a series of animal experiments in which the fate in the body of absorbed mercury was studied. In the discussion which follows, the results of this work, as well as other pertinent reports in the literature, will be summarized.

Theoretical considerations

It is well known from biochemical studies that mercury has a strong affinity for several organic substances or ligands common in biological structures. Of special significance are the sulfhydryl groups, as they occur wi-

dely and bind mercury with a very high association constant (HUGHES, 1957). Available biochemical data (summarized by PASSOW *et al.*, 1961) relating to the pharmacological and toxic effects of mercury and its

compounds indicate that they interfere in the biochemical reactions of the cell and the cell membrane. It has also been shown *in vitro* that mercury in small concentrations inhibits a number of enzyme systems. It is reasonable to assume that there is some relation between the cell concentration of mercury and the degree of functional disorder which results. As the cell mercury concentration increases, cell function is disturbed. When this disturbance is of sufficient degree, the level of mercury can be then considered as the maximum allowable concentration for that cell. The degree of disturbance which constitutes this threshold is a matter of definition. One factor of importance here is the reversibility of the disturbance. Chromosome damage, for example, may be irreversible. In actual practice, however, our ability to discriminate degrees of functional disorder is rather limited. It seems practical to place the threshold at a point at which disturbances are detectable, which corresponds to the definition used for M.A.C. values and recommended by the Prague Symposium. Probably this threshold will vary with the type of cell and the differences in the threshold between two cell types will depend upon, for example, the tendency of mercury to bind ligands and form inert complexes, thereby becoming more or less neutralized. Similarly, it may be assumed that for every organ there is a threshold at which symptoms of functional disturbance appear, and that this is dependent on what sort of cells the particular organ con-

tains. How soon the highest tolerable concentration of mercury will be reached in an organ will depend upon the concentration to which it is exposed, and the rates at which uptake and elimination occur. At a given level of exposure, the threshold of functional disturbance is first reached in one or several organs, which may be designated the critical organ(s) for that exposure; the tolerance of the organs in question is then the limiting factor in deciding how much absorption of mercury is permissible. However, it is possible that in different kinds of exposure, a different organ or organs will be critical. For example, an organ characterized by a very rapid uptake and a relatively rapid elimination of mercury may, during a brief but severe exposure, rapidly reach its threshold value, while another organ showing sluggish uptake, pronounced retention and a relatively high sensitivity for mercury, will not accumulate enough mercury during the short duration of the exposure to reach its threshold of functional disturbance. At a lower level of exposure over a prolonged time, however, the threshold may never be attained in the first organ whereas it will be in the second, which in this case constitutes the critical organ.

To establish M.A.C. values for the human environment, one must know which organs accumulate and retain mercury, how much mercury is retained and how sensitive these organs are to mercury. Furthermore, the route by which mercury enters the body must be understood; as this is dependent

upon the physical and chemical properties of the mercurial, it is important to know if the offending agent exists as a gas or as dust, in which case the particle size is critical, or whether it is present in water or food. The factors determining the rate of body absorption will obviously vary in different cases. In the discussion which follows, only those factors which are decisive in determining the highest tolerable body burden of mercury will be considered.

A great deal of information about the body distribution and retention of mercury under different conditions has been collected by experimental work in the past. This will be referred to below. Very few experiments, however, have been performed to estimate the sensitivity of different organs to mercury load. Our knowledge of this is derived mainly from the signs and symptoms of chronic and acute poisoning in animals and man, but the literature contains very few quantitative data. As the clinical

picture in mercury poisoning has been thoroughly reviewed by several authors, e.g. SPIEGL (1957), BATTIGELLI (1960), it will not be reiterated here.

It is of special interest to determine whether an index of the degree of body mercury load can be obtained by measurement of the mercury concentration in body fluids, in tissues which can be readily biopsied, and in excreta. To be useful, an index must reliably reflect the concentration of mercury at least in the critical organs. From the points of view of clinical diagnosis and of occupational health, it is also desirable to have an indicator of the past history of exposure to mercury, as well as of the present organ concentrations. It has already been mentioned that the excretion of mercury in the urine has been employed as an index of body content. The limitations of this indicator will be elaborated below, while the possibility of obtaining others which are more adequate will be considered.

BODY DISTRIBUTION AND CUMULATIVE RISK

INORGANIC MERCURY

Body distribution

The body distribution of mercury arising from administration of the element or its inorganic compounds has been studied with a number of analytical techniques. The earliest used of these were chemical, by which it was shown that mercury accumulates in the kidney, liver, spleen, intestine and myocardium (references to this work are given by BERLIN & ULLBERG, 1963 a). The largest deposits were invariably found in the kidney and after that, in the liver. The order of concentration in the other organs has not been reported with much consistency, no doubt as a result of differences in the mode of administration and dosage, as well as the relative insensitivity of the methods employed. More recently, by the use of spectrographic methods or of radioactive isotopes, mercury in small amounts has been demonstrated in other organs, for example in the brain (GRIFFITH *et al.*, 1954; FORBES *et al.*, 1954; HARRIS *et al.*, 1954) after its administration in subtoxic doses.

The aforementioned determinations were made on whole organs or large parts of them. When a histochemical technique or autoradiography was applied, however, it was shown that the distribution of mercury in the

kidney (ALMKVIST, 1903; VOIGT, 1958; BERGSTRAND *et al.*, 1958) and liver (FRIBERG *et al.*, 1957) is not uniform but rather quite differentiated. In the kidney, mercury was largely concentrated in the proximal tubules and in the liver, in the periphery of the lobules. BERLIN & ULLBERG (1963 a) made autoradiographs of 20 μ sagittal whole-body sections of mice given a single intravenous dose (0.5 mg mercury/kg body weight) of a highly active (1.5 C/g mercury) preparation of radiomercury. The picture of body mercury distribution so obtained (Figs. 1 and 2) emphasized the wide variation in concentration among and within the various organs and tissues. In addition to the organs enumerated above, mercury was also demonstrated in parts of the brain, in the skin, in the mucosa of the mouth, upper respiratory and alimentary tracts, and in bone marrow, in the interstitial tissue of the testes (Fig. 3), in the pancreas and salivary glands. In the brain (Fig. 4), an especially high concentration was found in the cerebellar cortex, parts of the hypothalamus, and the area postrema (in the posterior part of the floor of the fourth ventricle), the latter showing the highest concentration. The autoradiograms also confirmed earlier reports that mercury is excreted



Fig. 1 Autoradiograms of sagittal whole-body sections of mice injected with $Hg^{203}Cl_2$ (0.5 mg Hg/kg body weight), taken 20 min, 4 days, and 16 days after the injection, from BERLIN & ULLBERG (1963 a). The autoradiogram of the isotope reference scale accompanying the 20 min section is shown. The radioactive intensity ratio between successive steps is $1/2$.

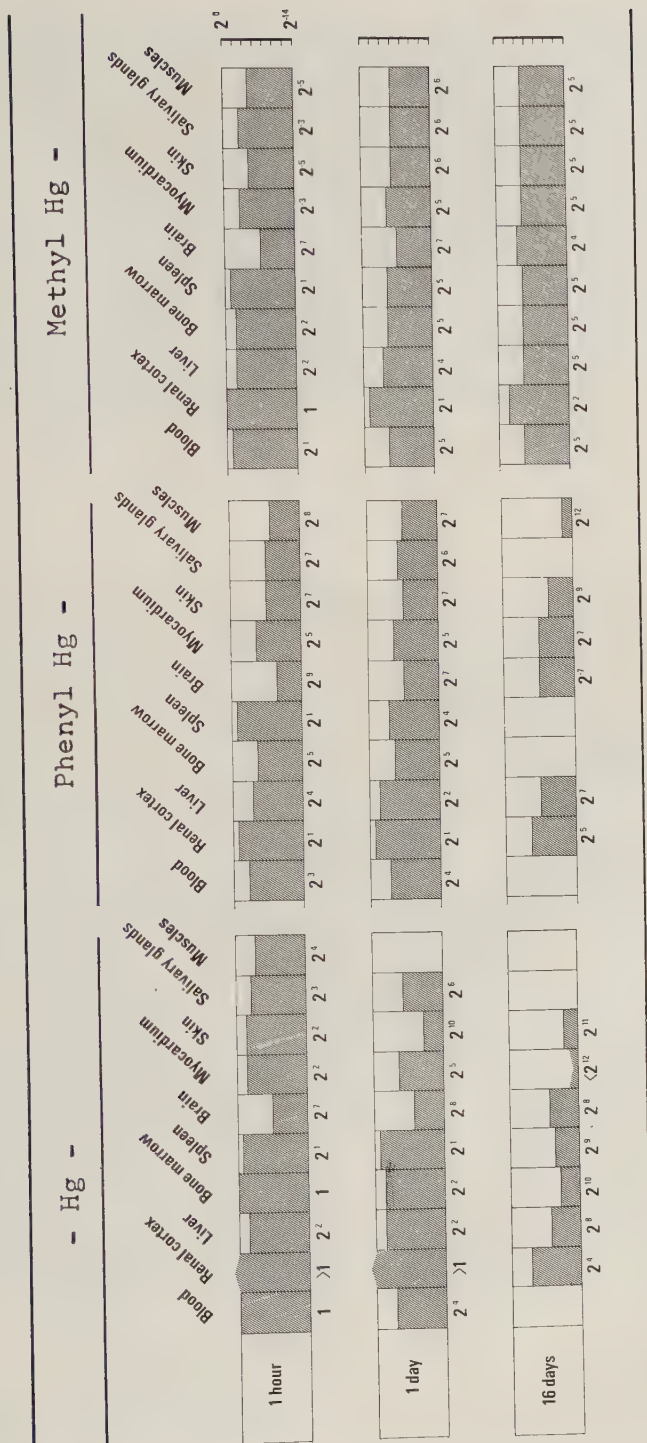


Fig. 2 The distribution of mercury among different organs at different times after single injections of 0.5 mg Hg/kg body weight as mercuric chloride (-Hg-), phenylmercuric acetate (Phenyl Hg-) and methylmercuric diguanidamide (Methyl Hg-), respectively, redrawn from BERLIN & ULLBERG (1963 a, b, c). The highest concentration in each organ, measured by optical densitometry against an isotope reference scale, is depicted as a vertical bar on a geometric scale (common ratio $1/2$) and is also given in arbitrary units as a power of 2.

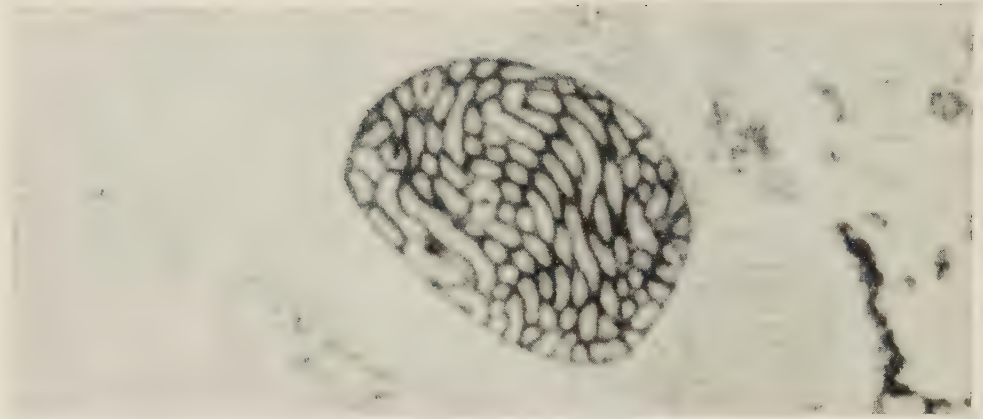


Fig. 3 An autoradiogram of a section of testis, 16 days after a single injection of $\text{Hg}^{203}\text{Cl}_2$ (0.5 mg Hg/kg body weight), in a mouse studied by BERLIN & ULLBERG (1963 a).



Fig. 4 An autoradiogram of a sagittal section of the brain of a mouse, 16 days after a single dose of 0.5 mg Hg/kg body weight as $\text{Hg}^{203}\text{Cl}_2$, from BERLIN & ULLBERG (1963 a). Note the dense accumulation in the area postrema, which here lies just behind the caudal tip of the cerebellum, in the hypothalamus just anterior to the light field corresponding to the hypophysis, and in tissue adjacent to the ventricles. The olfactory lobe is also prominent in this section.

in bile, and into the colon. Autoradiograms of pregnant mice near term (18 days) revealed that very little mercury was transmitted to the foetus but that a great deal was accumulated in the placenta.

Risk of cumulative effects

The risk of mercury accumulation in the cells and tissue of the body is dependent on the relation between uptake and elimination. The rates of these processes have, on the whole, received little study. FRIBERG (1956), however, exposed rats to mercury for a prolonged time by repeated subcutaneous injection of radioactively-labelled mercuric chloride. When exposure was discontinued and an attempt made to exchange the accumulated mercury with inactive mercury, large differences were found in the rate of exchange between radiomercury and inactive mercury in the kidneys, liver, spleen and brain. In the brain, no exchange was demonstrable during two weeks of trial. ROTHSTEIN & HAYES (1960) measured the retention of mercury in the rat after single intravenous or intramuscular injections of radiomercury at various times up to 48 days after injection. Their results showed differences in the rates of elimination among different organs, and they concluded that the greatest retention and risk of accumulation were to be found in the kidney. By densitometric comparison of the darkening in autoradiograms of whole-body sections of mice with that caused by appropriate isotope standards, BER-

LIN & ULLBERG (1963 a) were able to determine the order of mercury concentration within various organs at different times up to 16 days after a single intravenous injection of labelled mercuric chloride. This technique gave an estimate of the comparative rates of elimination among different organs or parts of organs. It was shown that those parts of the brain which took up mercury did so very slowly, and that after the maximum concentration was reached, some time between the fourth and the sixteenth post-injection day, no elimination was detectable from them. Nor was there seen any elimination of the mercury taken up by the interstitial tissue of the testes. In the oral mucosa and the skin, retention was pronounced and elimination slow. In the remaining organs, considerable elimination was observed once the maximal accumulation had been reached which, as a rule, occurred on the first day after injection. There is thus a considerable risk of accumulation of mercury in certain parts of the brain, testes, skin and oral mucosa because of the very slow elimination of mercury from these sites, while there is a similar risk in the liver and kidney if the degree of accumulation in them in comparison to other organs is considered.

Discussion and conclusions

In a previous paper (BERLIN & ULLBERG, 1963 a), it was pointed out that there is a conspicuous correlation between the site of accumulation of

mercury as found in the mouse by autoradiography, and the symptoms and signs of acute and chronic mercury poisoning. The occurrence of a dense accumulation of mercury in the renal cortex is fully consistent with the dominance of renal signs in acute poisoning. The colitis and other disturbances related to the intestinal tract, and the oral stomatitis which occur, also correspond to mercury accumulation in the mucosa of the different parts of the intestinal tract. The slow uptake and elimination of mercury observed in autoradiograms of the mouse central nervous system may also explain, to some extent, the symptoms which arise in chronic mercury intoxication in man, provided that similar conditions obtain in the human central nervous system. The dominant symptoms are referable to the central nervous system and often appear with a certain delay and persist for a long time after exposure is withdrawn. It remains for future microautoradiographic studies to reveal if the accumulation of mercury in the brain is limited to certain types of cells, which is not unlikely.

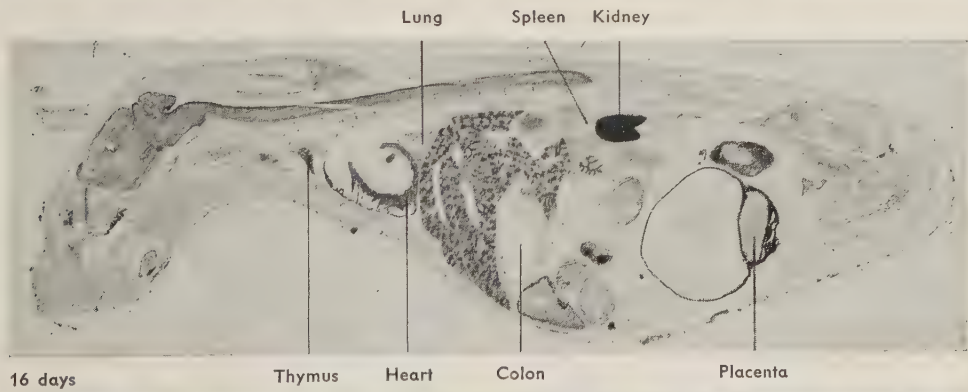
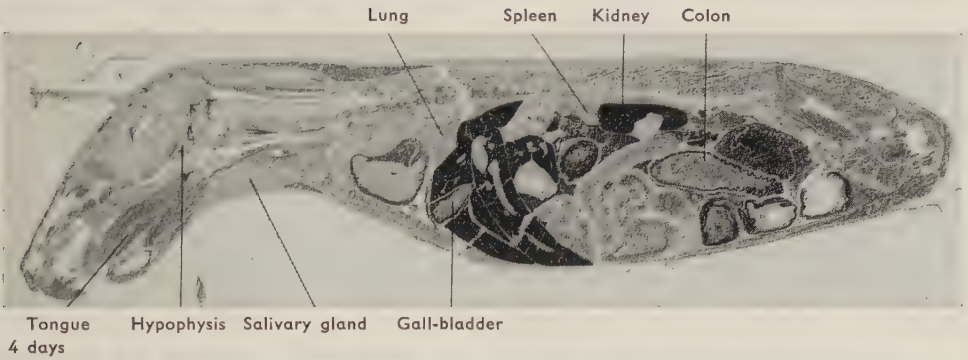
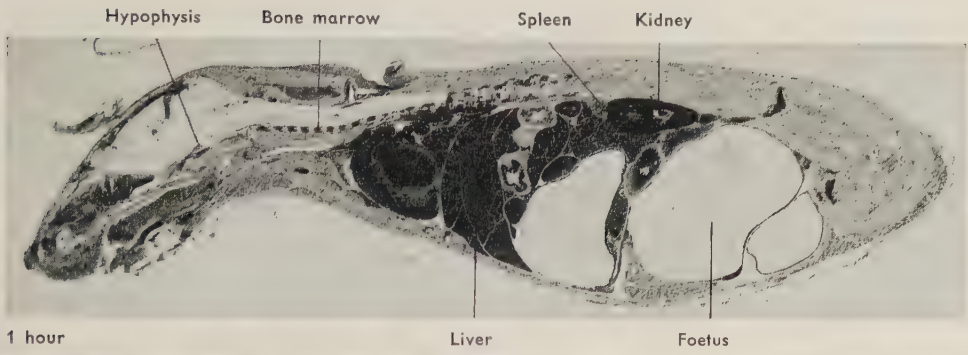
The distribution of mercury observed in the testes is very similar to that seen after cadmium exposure (BERLIN & ULLBERG, 1963 d). In cadmium poisoning, testicular atrophy, malformation of sperm and sterility have been observed (PARIZEK, 1960). Pathological changes of this kind in chronic mercury poisoning have not been reported but the accumulation and retention of mercury which have been found in the testes

suggest that further studies in this regard are warranted.

The commonest form of occupational exposure is to mercury vapour and most data referred to here are derived from studies in which mercury was given parenterally to experimental animals. However, the results obtained after the administration of mercury as a vapour (ASHE *et al.*, 1953) do not differ significantly from those obtained after parenteral administration. Moreover, CLARKSON *et al.* (1961) have clearly demonstrated that mercury vapour is readily oxidized in the blood to Hg^{++} , the process occurring mainly in the blood corpuscles. There is, therefore, no reason to believe that mercury entering the body as mercury vapour behaves differently compared to mercury injected in oxidized form.

It is reasonable to assume, on the basis of present knowledge, that the central nervous system and perhaps the testes are the critical organs in chronic exposure to subtoxic concentrations of mercury, while in acute exposure to high concentrations, the kidney is probably the critical organ. It is possible, however, that there is some tissue in the body which is very sensitive to small doses of mercury, but which does not show any marked accumulation even though serious

Fig 5. Autoradiograms of sagittal whole-body sections of mice injected with phenyl- Hg^{203} -acetate (0.5 mg Hg/kg body weight), taken 1 hour, 4 days and 16 days after injection, from BERLIN & ULLBERG (1963 b). The autoradiogram of the isotope reference scale accompanying each section is shown. The radioactive intensity ratio between successive steps is 1/2.



disturbances in its function may occur. At the present time, however, there is no clinical evidence to suggest the presence of tissue so sensitive. It is of some importance that further studies be made to determine

the concentration of mercury in the central nervous system or its parts at which functional disturbances appear, and to elucidate the mechanism by which mercury is taken up and accumulated there.

ORGANIC COMPOUNDS OF MERCURY

Body distribution

If an organic radical is attached to the mercury atom, the biochemical properties as well as the toxicity of the latter are significantly altered. A striking illustration of this is found in the extensive work relating to the development of mercurial diuretics in the past half-century. Differences in toxicity and body distribution have also been shown for organic mercury compounds which are important in occupational health. PRICKETT *et al.* (1950) found different body distributions of mercury after injection of phenylmercuric acetate and of inorganic mercury: accumulation was greater in the liver and more mercury was excreted in the faeces in the case of the organic compound. FRIBERG (1959) using the rat, and SWENSSON *et al.* (1959) the dog, showed that mercury is excreted more slowly after administration of methylmercuric compounds than after inorganic mercury, and that the body distributions differed as well. FRIBERG found more mercury in the brain after injection of a methylmercuric compound, while the mercury accumulation in the kidney was less than after inorganic mercury.

BERLIN & ULLBERG (1963 b and c) using the autoradiographic technique referred to above, studied the mercury distribution after a single intravenous injection of labelled aryl and alkyl compounds of mercury (phenylmercuric acetate and methylmercuric dicyandiamide, respectively). The dose in each case was 0.5 mg mercury/kg body weight. The distribution after injection of phenylmercuric acetate (Fig. 5) was in several respects very similar to that found after injection of inorganic mercury but certain differences were noted. In the first 24 hours after injection of phenylmercuric acetate, there was more accumulation in the liver and in the alimentary tract, but less in the renal cortex, than after inorganic mercury. These differences tended to disappear with time and, after 16 days, the distribution in the two cases were nearly identical. In the bone marrow and in the spleen, however, there was less retention in the case of phenylmercuric acetate.

Fig. 6 Autoradiograms of sagittal whole-body sections of mice injected with methyl-Hg²⁰³-dicyandiamide (0.5 mg Hg/kg body weight), taken 1 hour, 1 day and 8 days after injection, from BERLIN & ULLBERG (1963 c). The autoradiogram of the isotope reference scale accompanying each section is shown. The radioactive intensity ratio between successive steps is 1/2.

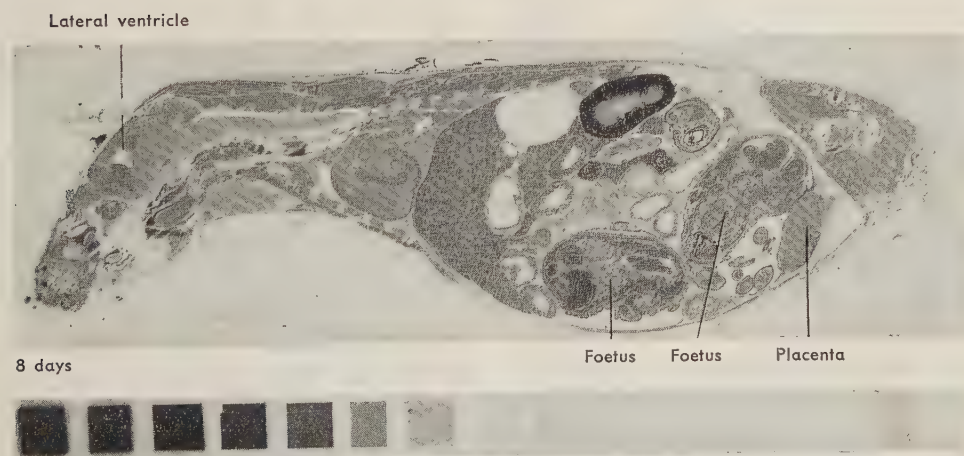
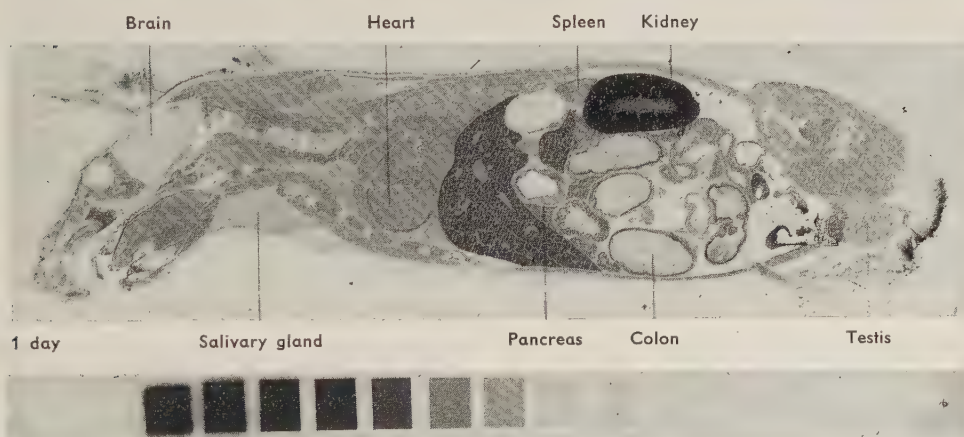
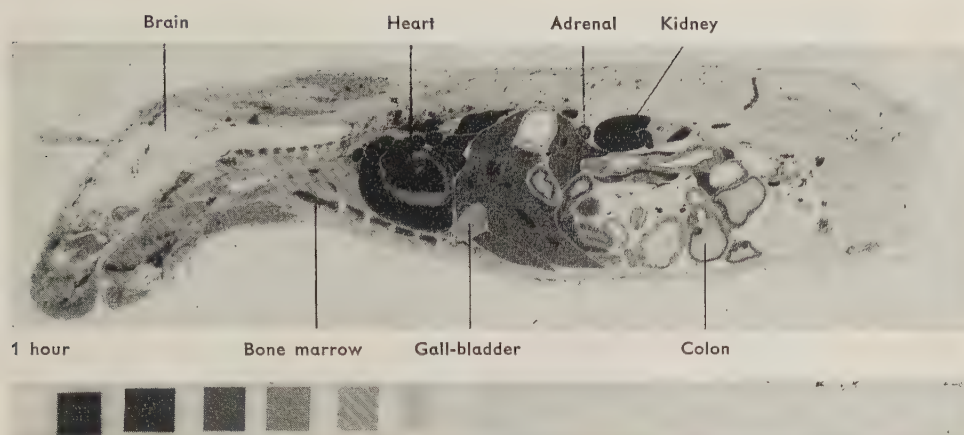




Fig. 7 An autoradiogram of a sagittal section of the brain of a mouse 16 days after a single dose of 0.5 mg Hg/kg body weight as methyl- Hg^{203} -dicyandiamide, from BERLIN & ULLBERG (1963 c). Note the dense accumulation in the hippocampus.

On the other hand, the injection of methylmercuric dicyandiamide resulted in a body distribution of mercury (Fig. 6) which was very different from that seen after injection of inorganic mercury. There was a rather uniform distribution which extended to the foetus in the pregnant mouse. Excretion of mercury could be observed in the bile and in the urine. In the first 24 hours after injection, the renal cortex and the liver accumulated more mercury than other organs, while very little mercury was seen in the brain and bone tissue. Afterwards, the mercury concentration in the liver tended with time to equal that of most other tissues in the body,

while mercury slowly accumulated in the brain to reach a maximum between the eighth and the sixteenth day after injection. At that time, the brain, (Fig. 7), especially the hippocampus and the cerebellar cortex, showed a mercury concentration which was exceeded only by the renal cortex among the other body organs.

Risk of cumulative effects

The rate of elimination of mercury after injection of phenylmercuric acetate was found to be the same order of magnitude in most organs as in the case of injection of inorganic mercury, as judged from mouse-section autoradiograms (BERLIN & ULL-

BERG, 1963 b). The risk of accumulation in the brain should then be at least the same in exposure to phenylmercuric compounds as in exposure to inorganic mercury, while the risk must be considered greater in the liver, which has the higher uptake, and smaller in the kidney, which has the lower uptake, in the case of phenylmercuric acetate. In exposure to methylmercuric compounds, the risk of accumulation in the whole body is of quite another order than that after injection of inorganic mercury, as shown by FRIBERG (1959) and SWENSSON *et al.* (1959). The very slow uptake and the dense accumulation of mercury in the brain demonstrated autoradiographically (BERLIN & ULLBERG, 1963 c) are of special interest in view of clinical experience in methylmercuric poisoning, as neurological symptoms are found to persist for a long time after exposure ceases. Why the maximum accumulation of mercury in the brain occurs so late in relation to other tissues in the body remains unexplained.

Discussion and conclusions

It was expected from biochemical considerations that the body distribution of mercury after the injection of organic mercurials would differ from that after injection of inorganic mercury, provided that the organic compounds persist as such in the body for a significant time. The rather similar distributions found after administration of phenylmercuric acetate and inorganic mercury make it likely that the phenylmercuric group

is broken down in the body. Such an interpretation is supported by the results of GAGE & SWAN (1961), who showed that inorganic mercury was excreted in the urine after injection of phenylmercuric compounds, and also by the results of MILLER *et al.* (1960), who found as inorganic mercury most mercury retained in the body one day after injection of a phenylmercuric compound.

The affinity of the methylmercuric radical for SH-groups is well established. On the basis of this property and the solubility of the methylmercuric derivatives, which renders them more diffusable through biological membranes, HUGHES (1957) postulated a uniform body distribution of mercury. The mercury distribution found in the mouse is consistent with this hypothesis and makes it unlikely that the carbon-mercury bond is broken in the body to any great extent.

The differences in distribution and elimination rate between the organic mercurials which have been studied confirm the expectation that it is not justified to look upon the organic compounds of mercury as a homogeneous group when deciding the highest tolerable body burden of mercury for each of them. Rather, it is necessary to consider the biochemical, pharmacological and toxicological properties of each compound in estimating the tolerable body burden. No evidence has appeared which would justify a lower value for the body burden of mercury in exposure to phenylmercuric compounds than in exposure to inorganic mercury. The lower acute toxic-

city which has been shown for phenylmercuric compounds may be due to the fact that a large part of the absorbed mercury is retained in the blood and that the acute load in the kidney is small compared to what it is after exposure to inorganic mercury. The central nervous system seems to be the critical organ in chronic exposure to low concentrations of phenyl-

and methylmercuric compounds. It is also probable that for both these organic compounds, disturbances in kidney function can appear in chronic exposure. It is therefore important that the mercury load in the brain, kidneys and liver at which disturbances can be detected after exposure to organic mercury compounds, be determined by future investigation.

DIAGNOSTIC INDICES OF EXPOSURE TO, AND RETENTION OF, MERCURY

The urinary excretion of mercury

INORGANIC MERCURY

Although it has long been known that mercury is accumulated in the kidneys and also excreted in the urine, little is known of the renal mechanisms of uptake and excretion of mercury. If the accumulation of mercury in the kidney is a part of the process of its excretion, then the urinary excretion of mercury would be a good index of retention in the body, since the kidneys are among those organs which retain mercury for the longest time. As mentioned above, several authors have used the urinary excretion of mercury as an index of exposure to, and accumulation of, mercury in man. However, the correlation between clinical findings and the urinary excretion of mercury is not convincing. TURRIAN *et al.* (1956) made a special study of this problem and could not find any correlation between the two. In experiments with rats, FRIBERG (1956) found pronounced variations in the daily mercury excretion in the urine during a constant exposure to mercury. ROTHSTEIN & HAYES (1960) studied the retention of mercury in the rat after a single injection by whole-body scintillation counting and also determined the mercury excretion in the urine. Their results do not support the view that there is a direct corre-

lation between mercury excretion in the urine and retention in the body. By using a method (BERLIN, 1962 a, b, c) which permitted, in the rabbit, the continuous measurement of radioactive mercury in urine and in blood entering and leaving the kidneys, (tongue), in the mucosa of the upper BERLIN & GIBSON (1963) determined the renal extraction, renal uptake and urinary excretion of mercury, simultaneously with the measurement of the glomerular filtration rate by creatinine clearance. These measurements were made while the blood concentration of mercury was held constant by adjustment in the rate of infusion of labelled mercuric chloride. During the four hours in which measurements were made, less than 10 % of the mercury in the blood was found to be extracted by the kidneys, and while up to 50 % of the infused mercury was accumulated in the kidneys, only a few per cent were excreted in the urine. The results indicated a correlation between the blood concentration and the urinary excretion of mercury, at blood concentrations of the order found in occupational exposure to mercury (about 100 micrograms/100 ml). When plasma was ultrafiltered, less than 1 % of the plasma mercury was found in the filtrate. It

therefore seems unlikely that plasma mercury passes through the glomerular membrane. The distribution of mercury between blood corpuscles and plasma was also studied during rising and falling blood concentrations of mercury. Variations in plasma and cell concentrations of mercury were out of phase, indicating a slow turnover-rate between the two compartments, each of which contained about half the blood total. These findings confirm the work of CLARKSON *et al.* (1961), who, in an independent study, arrived at the same results with respect to the ultrafiltrability of mercury and the distribution of inorganic mercury between corpuscles and plasma.

The accumulation of mercury in the kidney can take place either directly from the blood or through tubular reabsorption of mercury. This problem was studied in rabbits by measuring the uptake of mercury in both kidneys, the ureter of one of which was ligated to prevent glomerular filtration, (BERLIN & GIBSON, 1963). It was shown that in spite of the fact that filtration in one kidney was prevented, the uptake of mercury was essentially the same in both, and it was concluded that mercury which accumulates in the kidney is for the most part derived directly from the blood.

All evidence indicates that mercury is excreted in the urine through tubular excretion and that the rate of excretion is related to the blood concentration of mercury. It is likely that the accumulation of mercury in renal tissue takes place independently of the process of mercury excretion and probably results from combination with ligands in the renal cells.

Conclusions

The evidence presented above does not support the view that the urinary excretion of mercury can serve as a reliable index of the accumulation of mercury in the body. Mercury excretion in the urine reflects the concentration of mercury neither in the kidneys nor in the brain. It is possible, however, considering the rather strong correlation between blood concentration of mercury and urinary excretion of mercury (BERLIN & GIBSON, 1963) that the urinary excretion of mercury could give an indication of the amount of mercury to which the body has been exposed in the very recent past. Nevertheless, experimental differences in the rate of excretion after different doses of mercury make it doubtful whether there is any linear relationship between the rate of excretion and the level of exposure.

ORGANIC COMPOUNDS OF MERCURY

It is known from investigations with mercurial diuretics that the combination of an organic radical with the mercury atom alters the rate and

mode of mercury excretion in the kidney. Of the organic mercuric compounds which are of interest in occupational health, the phenyl- and me-

thylmercuric compounds have been studied by PRICKETT *et al.* (1950), FRIBERG (1959) and SWENSSON *et al.* (1959). It has been shown that the rate of mercury excretion is different after administration of each of these compounds and inorganic mercury. This is especially clear in the case of methylmercuric compounds, which are excreted very slowly in the urine. There is evidence that phenylmercuric compounds are broken down in the body and are in large part excreted as inorganic mercury (GAGE & SWAN, 1961). The technique referred to above which was used in studying the excretion of inorganic mercury has been applied to the problem of mercury excretion after infusion of methylmercuric dicyandiamide and of phenylmercuric acetate (BERLIN, 1963). It was found that about 90 % of the blood mercury was bound to the corpuscles after infusion of each of these mercurials and that less than 1 % of the mercury in the plasma could be ultrafiltered. It was calculated that for equal plasma mercury concentrations, the mercury excretion after the phenylmercuric compound was of the same order as that after infusion of inorganic mercury, while the excretion of mercury after infusion of the methylmercuric compound was about ten times less. In the case of both organic compounds and inorganic mer-

cury, there was considerably more mercury accumulated in the kidneys than was excreted in the urine. The rate of urinary excretion of mercury after infusion of the organic compounds was also related to the blood concentration of mercury.

Conclusions

The mechanism by which mercury is excreted in the urine after exposure to the aryl and alkyl mercury compounds which have been studied does not appear to differ in principle from that after exposure to inorganic mercury. In the case of the phenylmercuric compound, the excretion of mercury reflects neither the body burden of mercury nor the concentration in the critical organ. In the case of the methylmercuric compound, the blood concentration of mercury, judged from autoradiographic investigations (BERLIN & ULLBERG, 1963 c), is a better reflection of the body burden of mercury, and hence in this case, the renal excretion of mercury may be more significant of the body total. However, as the time course of mercury accumulation in the brain is out of phase with that of the rest of the body, it may not be a useful indicator of the retention of mercury in the brain, which must be considered as the critical organ in exposure to methylmercuric compounds.

Other Possible Indices

If the urinary excretion of mercury cannot serve as an index of the accu-

mulation of mercury in the body, can the mercury content of any other ex-

cretion product or any tissue be used in this regard? The following requirements must be fulfilled by a suitable index. The specimen used for analysis must be easily obtained, with little or no discomfort or risk to the patient. Its mercury content must be equal, or reliably related, to the mercury concentration in the critical organs. It is then natural to seek a tissue which accumulates mercury to the same degree as the brain or the testes and is accessible to biopsy. It is unlikely that other excreta such as faeces or saliva, although readily obtainable, can fulfill all requirements (BERLIN & ULLBERG, 1963 a). In the case of inorganic mercury, mouse autoradiograms (BERLIN & ULLBERG, 1963 a) showed accumulation of mercury in the skin, in the epithelium of the mouth (especially the tongue), in the mucosa of the upper respiratory tract and colon. As activation analysis permits the determination with great accuracy of small amounts of mercury, it seems probable that mercury could be determined by this method in biopsies of epithelial tissue. If mercury accumulates in the human skin to the same degree as in the mouse, this tissue would be most suitable for biopsy and mercury determination. While the possibility that the skin may absorb mercury directly from the air or other contact has to be considered, there may be skin surfaces which are adequately protected. Scrapings from the plantar surface or toe-nail clippings may be suitable. Scrapings from

the tongue or biopsies of the upper respiratory epithelium may also be used, but most workers are exposed to mercury vapour and therefore the mercury concentration at these sites may not reflect that in the critical organ. In hospitalized patients, biopsies from the kidney, liver or colonic mucosa may be employed to establish a correct diagnosis. In the case of the organic mercury compounds discussed here, there is no reason to assume any major differences between phenylmercuric compounds and inorganic mercury when considering possible indices for the accumulation of mercury in the body. In the case of methylmercuric compounds, the period studied after the exposure in most investigations has been too short to permit any conclusions about the differences in elimination rate from different organs. It may be that in this case the blood concentration of mercury and also the mercury excretion in the urine can serve as suitable indices.

Conclusions

Present knowledge of the distribution and risk of accumulation of mercury in different organs of the body indicates that it would be worthwhile to study, in victims of chronic exposure to mercury, the mercury content in scrapings of the skin and tongue, in toe-nail clippings, and in biopsies of kidneys, colonic mucosa and liver and to correlate the findings with clinical signs of mercury intoxication.

SUMMARY

A brief historical review of the study of occupational exposure to mercury is presented and the basis for the present M.A.C. values of mercury compounds is examined. Important factors in the determination of the tolerable body burden of mercury are discussed, notably the body distribution of mercury after exposure, and the risk of cumulation in different organs. In acute exposure the kidney and liver accumulate much mercury and are hence liable to injury, while recent findings indicate that in

chronic exposure to moderate levels of mercury the brain and possibly testes are the critical organs because of a pronounced tendency to cumulation. The possibility of obtaining an index of mercury retention is explored; it is concluded that urinary mercury excretion does not reflect the level of body retention although it may indicate very recent exposure. It is suggested that mercury concentration in biopsies of skin, liver, kidney and colonic mucosa may serve as an index of body retention of mercury.

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